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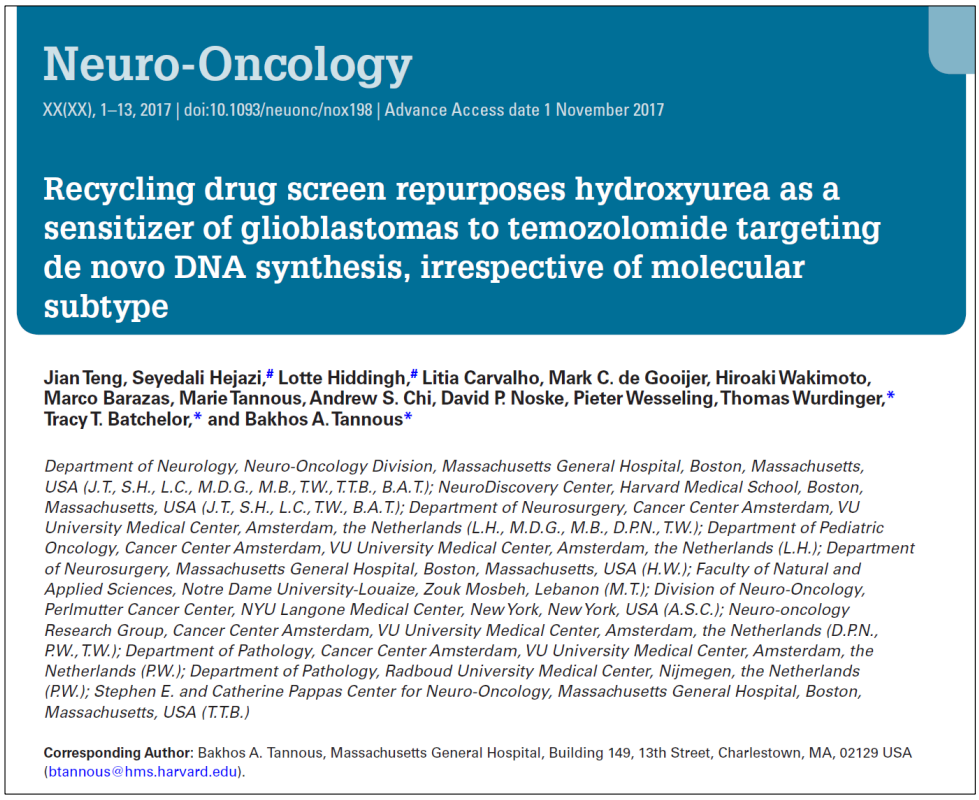
Hydroxyurea combined with temozolomide for treating glioblastoma



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Recycling drug screen repurposes hydroxyurea as a sensitizer of glioblastomas to temozolomide targeting de novo DNA synthesis, irrespective of molecular subtype

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Lay summary

Although chemotherapy of brain tumours with temozolomide (TMZ) has improved glioblastoma patient survival compared with earlier therapies, the development of resistance to this drug is a major problem. This article describes the search for drugs that may overcome this resistance when used in combination with TMZ. Hydroxyurea was identified as such a drug and shown to significantly enhance the killing of a range of GBM cell types. The detailed findings are sufficiently encouraging to support an early clinical trial of hydroxyurea and temozolomide in patients.

Background

The DNA alkylating agent temozolomide (TMZ) is used routinely for the treatment of primary glioblastomas (GBM) but provides only a modest increase over an already short life expectancy from initial diagnosis [1]. The reason for this is the development of acquired drug resistance to temozolomide within the tumour and the consequent outgrowth of malignant cells. This problem has been the focus of some of our *Window on Glioblastoma* articles, mostly in relation to the stem cell types that give rise to drug resistance. In this article, we report on a molecular mechanism for TMZ resistance and how this has been exploited by Teng et al.[2] to identify the FDA-approved drug hydroxyurea as a potential adjuvant therapy for GBM. Their work is also the subject of a patent application on behalf of the authors' institution, the Massachusetts General Hospital [3].

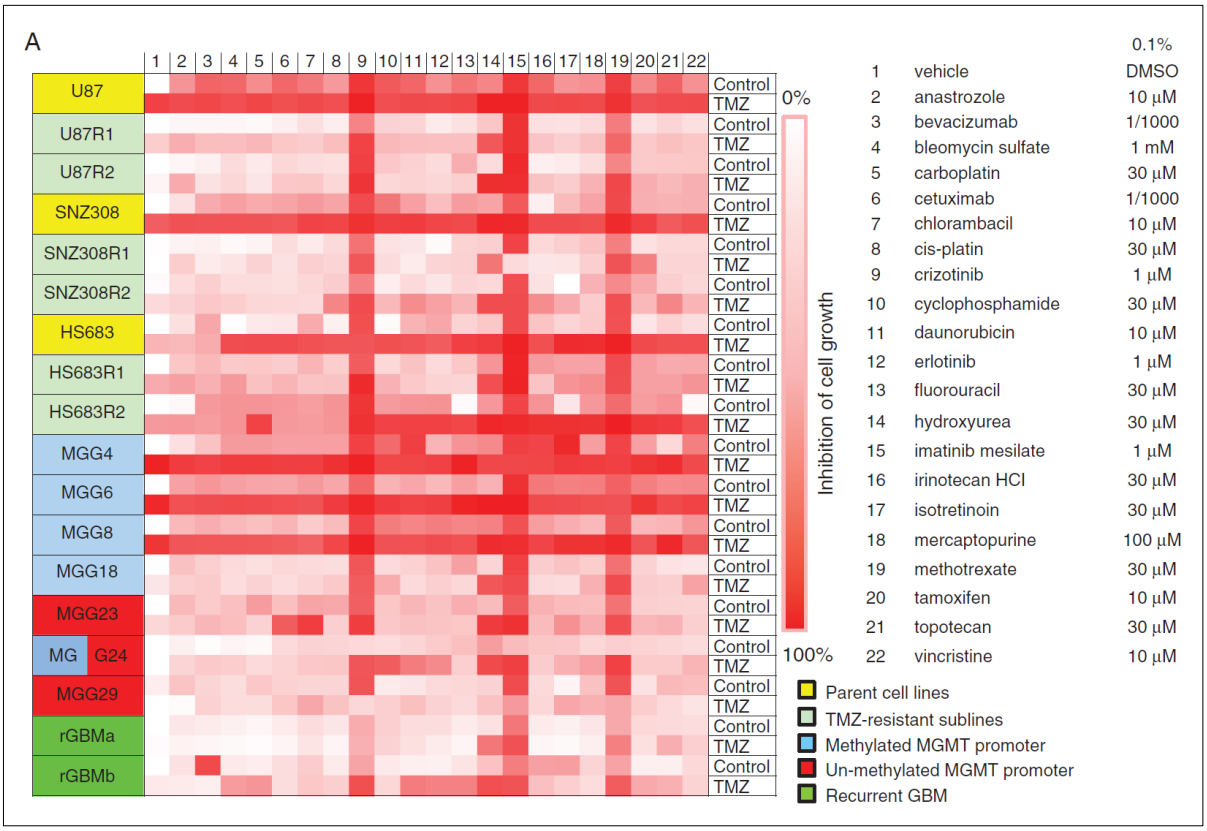
Mechanisms of resistance to TMZ

TMZ methylates DNA at the O6 position in guanine leading to impaired DNA repair and ultimately apoptosis. The O6-methylguanine DNA methyltransferase (MGMT) removes the DNA adduct caused by this alkylating agent resulting in resistance to TMZ therapy[4], with DNA methylation on the MGMT promoter being a major predictor. Another resistance mechanism involves loss of function in the MutS homolog 6 (MSH6) mismatch repair gene [5]. Taking the above findings into consideration, Teng et al.[2] reasoned that it should be possible to identify compounds that overcome resistance by interfering with specific resistance pathways and thus be used as an adjuvant with TMZ for treating brain tumours.

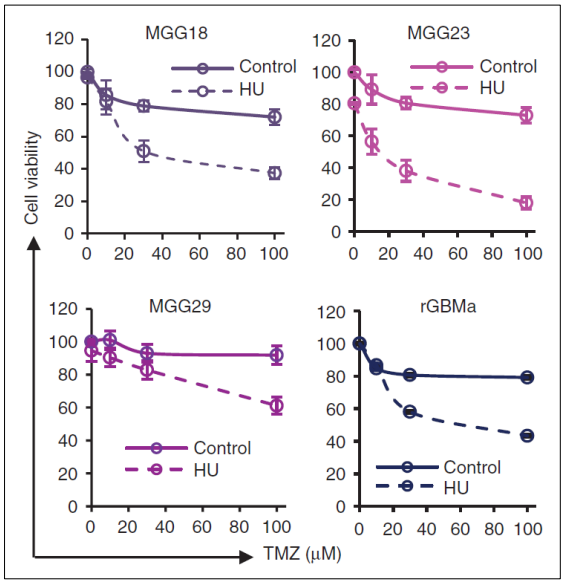
Hydroxyurea interferes with resistance to TMZ

FDA-approved drugs in current use for oncology were used to screen a panel of GBM cell lines, some of which had been selected for resistance to TMZ [2]. The cells were both standard GBM lines grown in standard media (such as U87), as well as neurosphere cultures derived from GBM explants.

The heatmap below, taken from [2], shows the effect of 21 commonly prescribed chemotherapeutic drugs on cell viability after 72 hours with and without TMZ (white no inhibition of cell growth, red complete inhibition). Hydroxyurea (HU) at 30 micromolar affected all cell lines, with a single exception, so this compound was chosen for further evaluation.

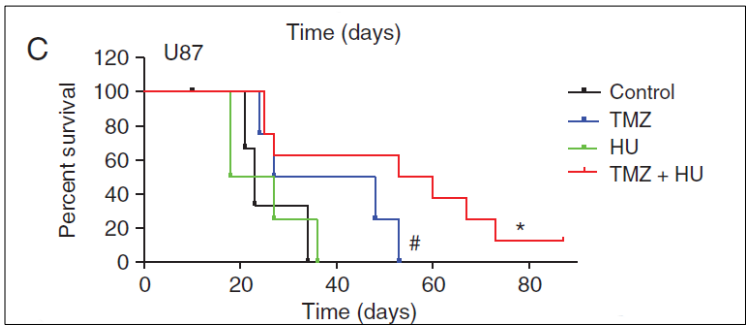


The panel below shows the viability of some cell lines treated with 30 micromolar hydroxyurea in the presence of increasing amounts of TMZ. These *in vitro* studies were extended using different cell lines to measure IC50s of the effect of HU on cell viability in the presence of TMZ, as well as a proapoptotic effect using annexin V/propidium iodide staining and flow cytometry.



In vivo effects of HU on TMZ resistance

An orthotopic mouse model was used to measure the viability of U87 cells *in vivo*. Using a lentivirus vector, cells were transfected with firefly luciferase (Fluc) as a reporter system for direct *in vivo* bioluminescent imaging using a Xenogen IVIS 200 system [6]. 50,000 U87 GBM cells were implanted in the brain striatum and the mice dosed with HU and TMZ, alone or in combination, to assess changes in tumour growth and animal survival. The Kaplan Meir survival curve below (taken from Figure 2 of Teng et al.[2]) shows the increase in survival afforded by HU in conjunction with TMZ.



To produce a more realistic clinical scenario, a large number of U87 cells were implanted and left to form a larger tumour, after which radiotherapy was included along with TMZ and HU treatment. Again, both drugs significantly improved survival, which was enhanced further with the radiation treatment (Figure 2L in the paper).

Effectiveness of the HU/TMZ combination in different GBM backgrounds

If the adjuvant effect of HU is to be clinically useful, it must work on all the cellular phenotypes actually found in glioblastoma. This includes those with methylated MGMT promoters, and those with stem cell, classical or mesenchymal phenotypes. This was shown to be the case through a series of *in vivo* experiments described by Teng et al.[2].

Having established that the HU/ TMZ combination could be developed for clinical trials, the authors conducted a preliminary toxicity assessment in mice (dosing 4 days/week for 2 weeks). There was no significant difference between control and treatment groups in terms of white blood cells, haematocrit, red blood cells, mean corpuscular volume, red cell distribution width, haemoglobin, mean corpuscular haemoglobin, platelets, and mean platelet volume.

Lastly, HU will penetrate the blood brain barrier [7] and furthermore, can enhance the uptake of certain chemotherapeutics by means of nitric oxide production [8]. If HU increases TMZ penetration into the brain and/or GBM tumours, it could potentially widen the therapeutic window of TMZ by decreasing the dose required to give a therapeutic effect.

Mechanism of action of the HU/TMZ combination

Hydroxyurea is used in oncology because it inhibits ribonucleotide reductase M2 (RRM2), an enzyme involved in nucleotide metabolism[9]. The paper by Teng et al.[2] also demonstrated that reduction of RRM2 expression in cell lines by transfecting them with a specific shRNA mimicked the enhancing effect of HU on sensitivity to TMZ. This was further corroborated using the ribonucleotide reductase inhibitors fludarabine and gemcitabine.

Conclusions

From the work of Teng et al.[2] outlined here, and the patent application [3] based on the published findings, a Phase I clinical trial of hydroxyurea and temozolomide in combination for treating GBM seems to be warranted. As with all the promising experimental therapies described in these *Window on Glioblastoma* articles, and in the wider literature, there are caveats. Teng et al.[2] include in their discussion the following: “In this study, we observed tumor regrowth around 2 weeks after the 2-week treatment window was discontinued. It would be of great interest to evaluate whether longer treatment period might give better therapeutic outcome as well as mechanism of resistance to TMZ/HU combined therapy in future studies.”

Nevertheless, clinicians treating GBM patients now have considerable experience with TMZ chemotherapy, so hopefully this expertise will help in the design of new combination treatments involving hydroxyurea, RNR inhibitors or indeed any other drug that overcomes resistance to this alkylating agent.

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